

# OSTEOARTHRITIS and CARTILAGE

## Release of mast cell mediators and nitrites into knee joint fluid in osteoarthritis—comparison with articular chondrocalcinosis and rheumatoid arthritis

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### Summary

In order to address the issue of the role of mast cells and nitric oxide (NO) in joint effusions occurring in the course of osteoarthritis (OA), synovial fluids collected from the knee of patients with OA, articular chondrocalcinosis and rheumatoid arthritis (RA) were studied for number of mast cells, and histamine, tryptase, phospholipase A2 and nitrite content. Mast cell counts are elevated in synovial fluid from OA patients when compared with RA. Histamine content in synovial fluid parallels the number of mast cells. Tryptase levels are elevated in OA in comparison with both other conditions, but do not reach the level of significance. Identical phospholipase A2 levels are recorded in three groups. Nitrite concentrations are also higher in synovial fluid from OA patients when compared with RA patients. These results suggest that mast cells in association with various inflammatory cells, may contribute to inflammation and cartilage breakdown in OA.

**Key words:** Osteoarthritis, Mast cells, Tryptase, Nitrites.

### Introduction

CLINICAL features of osteoarthritis (OA) may vary according to mechanical, metabolic or environmental conditions constituting risk factors in an individual patient. Despite the fact that the initial alteration in OA can be located in joint cartilage, changes of subchondral bone, osteophytosis and synovial inflammatory reactions are important in the course of the disease and are produced as a consequence of the action of various mediators. Articular chondrocalcinosis (ACC) is characterized by calcium crystal deposits in cartilage tissue.

Cartilage breakdown can be related to the secretion of abnormal matrix components by dysregulated and mutant chondrocytes. Degrading substances, such as metalloproteinases also contribute to cartilage alterations [1].

Interactions between synovial membrane cells and cartilage are extremely important for explaining the occurrence of cartilage alterations and flares in the course of OA. Production of interleukins (IL), especially IL-1 and TNF- $\alpha$  by synovial cells is well documented, and these

mediators can activate chondrocytes as well as synovial cells [2].

During the course of the disease, a great number of OA patients suffer from joint effusions, especially in the knee, with pain and disability, and often require a treatment with intra-articular corticosteroid injections.

The presence of mast cells (MC) has been recorded in synovial fluid (SF) or synovial membrane, especially in rheumatoid arthritis (RA) [3-8]. However MC are less numerous than other cells such as polymorphonuclear cells, macrophages or lymphocytes. Indeed, this finding may be of importance in view of the recognized role of these cells in both acute and chronic inflammatory processes. Nevertheless, the exact role of MC in synovial inflammation in human disease is not yet elucidated. Upon activation either through the high affinity receptor for IgE immunoglobulin (Fc $\epsilon$  R1) or upon action of cytokines, MC release preformed mediators such histamine and the proteolytic enzyme tryptase. Newly synthesized mediators are also released, and phospholipase A2 (PLA2) activation is an important initial element of the biochemical cascade leading to the synthesis of arachidonic acid derived mediators.

Nitric oxide (NO) was recently identified as a potent mediator of inflammatory reactions, in

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view of its vasodilating properties. It is produced by many cell types including endothelial cells, macrophages, mast cells, neutrophil polymorphonuclear cells and platelets. It is rapidly degraded into nitrites and nitrates in biological fluids.

In order to address the issue of the role of MC and NO in the genesis of joint effusions in OA, SF from patients with OA, ACC and RA were studied for number of MC, histamine, tryptase, PLA2 and nitrite content.

## Patients and Methods

### SF

SF were obtained from the knee under sterile procedure during arthrocentesis or arthroscopy for diagnostic purposes, collected in heparinized tubes and centrifuged. A visual count of the cells using a hemocytometer was done to insure that the supernatant had been rendered cell-free. The supernatants were stored at  $-80^{\circ}\text{C}$ , and subsequently assayed for mediators.

The total number of patients was 51 (33 women and 18 men). The diagnosis of OA was made in 16 patients (nine females, seven males, mean age  $68.6 \pm 10.3$ ) on the association of joint space narrowing, erosions and osteophytes on X-rays, in the absence of clinical, biological or radiological signs suggesting an inflammatory rheumatism. The diagnosis of ACC was made in presence of calcium deposition in articular cartilage on X-rays and presence of calcium pyrophosphate crystals in SF by microscopic examination. Fourteen patients (eight women and six men, mean age  $76.3 \pm 15.4$ ) were included in this group.

The diagnosis of RA was made in 21 patients (16 women and five men, mean age  $57.5 \pm 6$ ) on the basis of American Rheumatism Association revised criteria for RA [9]. These patients were included in the study, as synovial effusions are frequently observed in this chronic joint disease with an immunological background. All RA patients had an active disease at the time of examination, and received a treatment by disease modifying anti-rheumatic drugs, along with non-steroidal anti-inflammatory drugs, and in some cases oral corticosteroids.

### MC ENUMERATION

Cyocentrifuge preparation of SF was made immediately after collection, fixed in methanol and stained with Alcian blue/safranin. In the first experiments, blue toluidine staining was also used and gave similar results than alcian/safranin

staining. The first 500 cells encountered in random high power fields ( $\times 50$ , oil immersion) were observed and counted. The proportion of MC was derived as a percentage of all nucleated cells.

### HISTAMINE

Histamine levels in SF were determined using the fluorometric method of Shore [10] modified with an automated continuous-flow technique [11]. The limit of detection was 500 pg/ml for samples of 230  $\mu\text{l}$ . The reproducibility was  $\pm 2\%$  for concentrations below 2 ng/ml. Results are expressed in ng/ml. Extensive tests for specificity are reported in [11].

In order to ascertain the absence of histaminase in SF, a known amount of histamine (1.2, or 4 ng/ml) was added to five histamine-free synovial fluids. The samples were incubated 3 h at  $37^{\circ}\text{C}$  and tested again for the presence of histamine. Histamine was detected in all the samples tested.

### TRYPTASE

Tryptase was assayed using a solid-phase radio-immuno-assay based on the use of an anti-human tryptase monoclonal antibody (Pharmacia Tryptase RIACT<sup>®</sup>, Kabi Pharmacia, Uppsala, Sweden) [12]. Results are expressed in U/ml.

### PLA2

Extracellular PLA2 activity was determined using the fluorometric method described by Radvanyi *et al.* [13] using as a substrate the 1-hexadecanoyl-2-(1-pyrenyldecanoyl)-sn-glycero-3-phospho-glycerol (Molecular Probes Inc., USA). The fluorescent phospholipid was suspended in ethanol at a concentration of 0.2 mM; vesicles were prepared by adding the phospholipid dissolved in ethanol to an aqueous medium. The reaction solution was prepared by adding sequentially 1 ml buffer containing 50 mM Tris-HCl pH 7.5, 100 mM NaCl, and 1 mM EDTA, 10  $\mu\text{l}$  of substrate (2 mM final concentration), 10  $\mu\text{l}$  of serum albumin solution (0.1% final concentration), 6  $\mu\text{l}$  of 1 M  $\text{CaCl}_2$  (6 mM final concentration).

The fluorescence of the reaction medium was recorded (as blank) and the reaction was initiated by the addition of the phospholipase. The activity in nm/min was calculated according to the formula:

$$A = 2 \times 10^{-4} \frac{(S - S^{\circ})V}{F_{\max}}$$

$S$  is defined as the slope of the curve representing the increase of fluorescence vs time.  $S^{\circ}$  is the slope

observed in the absence of the enzyme (blank).  $V$  is the volume ( $\mu$ l) of pyrene phospholipid (0.2 mM) in the reaction medium and  $F_{\max}$  the maximum fluorescence signal at the end of the reaction in presence of 5  $\mu$ g PLA2 from *Naja naja* (Sigma Chem., St Louis, MO, U.S.A.). All fluorescence measurements were done with a Jobin et Yvon JY three-dimension spectrofluorometer equipped with a Xenon lamp. The fluorescence intensity was monitored using excitation and emission wavelengths of 345 nm and 398 nm respectively. PLA2 activity is expressed as nmol/min/ml.

#### NITRITE

Nitrite concentrations were measured by a colorimetric method using the Griess reaction as described in [14]. The nitrite concentration is expressed in nmol/ml.

#### STATISTICS

Mean values of the biological parameters were compared between groups using the Wilcoxon's test.

#### Results

As expected, cell counts are significantly lower in SF from patients with OA or ACC than in SF from RA patients. MC counts are elevated in SF of OA when compared with RA. Histamine content in SF parallels the number of MC. The histamine concentration in SF is significantly higher in OA than in RA and in ACC ( $P < 0.05$ ). Tryptase levels are elevated in OA in comparison with both other conditions, but do not reach the level of significance. Identical PLA2 levels are recorded in three groups. Nitrite concentrations are also higher in SF from OA patients, compared with RA patients. (Table I).

#### Discussion

Pathophysiological mechanisms of inflammation and synovial effusion in OA are still uncertain, but MC are likely to play a role. Previous cytological studies of SF have shown that MC numbers are elevated in OA when compared with normal controls or with RA [8]. MC from OA synovia were reported to contain and release more histamine than MC from RA synovia [6].

This work aimed at further evaluating the role of MC in OA and in ACC by means of MC counts in SF and by assaying mediators released by these cells into the SF. In order to appreciate the relative role of various cells, assays were performed for histamine, a mediator released by MC and polymorphonuclear basophils, tryptase, a MC specific mediator, PLA2, an enzyme produced by various inflammatory cells, and nitrite, as a metabolic endpoint of nitric oxide. Interpretation of the results is made difficult by the lack of data concerning normal controls. Thus we chose to compare three pathological conditions, being conscious of the drawback of this method concerning the significance of the results.

If one considers the percentage of MC, our results are very comparable with those reported by Dean *et al.* [8]. These authors found 0.029% in RA, vs 0.03% in our patients, and 0.84% in established OA vs 0.2% in our patients. Nevertheless, they did not calculate the absolute number of MC in SF. Our results show that the absolute MC number in RA (4620 cells/ml) is superior to the MC number in OA (1340 cells/ml) because of the higher nucleated cell concentration in RA fluids. The results of histamine assay show that histamine concentration in OA fluid is elevated when compared with RA.

Several considerations may help to explain this apparent discrepancy. Enumerating MC either in biological fluids or on histological tissue sections bears an inevitable error, because of the fact

Table I  
Mast cell activation products and nitrites in synovial fluid

	Osteoarthritis N = 16	Chondrocalcinosis N = 14	Rheumatoid arthritis N = 21
Percentage of mast cells	20 $\pm$ 10	ND	3 $\pm$ 7
Total number of nucleated cells ( $\times 10^6$ /ml)	0.67** $\pm$ 0.9	2.8** $\pm$ 6.0	15.4 $\pm$ 9.8
Histamine (ng/ml)	10.4 $\pm$ 9.9*	8.7 $\pm$ 4.9*	5.1 $\pm$ 4.2
Tryptase (U/ml)	3.5 $\pm$ 13.3	0.35 $\pm$ 0.6	0.35 $\pm$ 1.4
Phospholipase A2 (nm/min/ml)	31.7 $\pm$ 13.3	22.5 $\pm$ 13.2	36.3 $\pm$ 21
NO <sub>2</sub> (nm/ml)	0.56 $\pm$ 0.3 (N = 6)	0.38 $\pm$ 0.2 (N = 6)	0 $\pm$ 0 (N = 12)

Results are given as mean  $\pm$  s.d. \* $P = 0.05$  vs RA. \*\* $P = 0.01$  vs RA.

that activated MC, i.e. MC which are liable to be responsible for the disease, are not accessible to the count because they are partially or totally degranulated, and consequently poorly or not stained. In our experience, MC enumeration in SF from ACC patients was not possible because of technical difficulties linked to the high viscosity of the material.

Histamine is generally considered a mast cell originated mediator, but polymorphonuclear basophils are also able to contribute to the histamine content of a biological fluid.

Tryptase is a proteolytic enzyme, which is specifically located in and released by MC. Among the different diseases here studied, OA was the only one in which tryptase was detected in significant levels. Because tryptase is normally absent in biological fluid and released by MCs upon action of various stimuli, the presence of this substance is in itself of significance. Furthermore, in the case of RA and ACC, several fluid samples were totally devoid of assayable tryptase. When histamine release is not accompanied with detectable tryptase levels, an occurrence observed in ACC, a possible role of polymorphonuclear basophils may be hypothesized, but the identification of these cells in the fluid by optical observation was made impossible by technical difficulties.

In OA, the presence of histamine and tryptase in SF at higher concentrations than in ACC or RA, despite the lower mean MC count, suggests the activation of these cells. Or alternatively the presence of inhibitors of tryptase or histamine in RA SF (which would cause problems with the measurement of these products), or the presence of proteases able to degrade them could account for the higher concentrations in OA compared with RA. However in our experiments, the detection of the histamine previously added to SF was not in favor of the presence of histaminase. The proteases are likely to be present also in OA SF. Further experiments will have to be done to clarify this issue.

The demonstration of MC activation products in SF raises the question of activation mechanisms. In view of the demonstration that MC can be activated or modulated by various lymphokines or cytokines, the role of these mediators appears likely [15–17].

On the other hand PLA2 assay yielded very similar results in three of the conditions studied, presumably because PLA2 activation develops in various cells and represents a feature of a common activation pathway occurring in inflammatory cell membrane and leading to platelet aggregat-

ing factor (PAF) and arachidonic acid derived mediators.

Nitrite levels were elevated in OA and in ACC fluids, suggesting that NO release is involved in synovial inflammation in OA. Further study is necessary to precisely identify the cellular origin of NO, but the presence of this mediator may help explain vasodilatation and fluid leakage into the joint. In contrast, nitrites were not detected in either serum or SF from RA patients. This result may correspond to nitrite levels below the detection threshold of the method, but an alternative explanation is that nitrite is masked by its incorporation into nitrosoproteins. A consequence of treatment activity is improbable, because most patients received the same drugs at the onset of the joint effusion, but cannot be excluded.

Our finding that MC activation products are detectable in significant amounts in SF in OA confirms and extends the results of Dean *et al.* [8], and suggest that histamine may contribute to the effusion of SF with a low cell content. The fact that tryptase is able to activate collagenase [18] confirms the interest of further studying the release of this proteolytic enzyme in rheumatic diseases. On the other hand, the recent demonstration that NO enhances apoptosis in chondrocytes [19] suggests that our finding of high nitrite levels may help explain the mechanisms underlying cartilage breakdown in OA.

In conclusion, our data confirm the presence of MC mediators and NO in SF in OA and suggest that these mediators could play a role in the events leading to the constitution of joint effusion during the course of OA.

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